

**VARIANT IgG3 RITUXAN® AND THERAPEUTIC
USE THEREOF**

Cross Reference to Related Application

[0001] This application claims priority to U.S. Serial No. 60/241,022, filed October 20, 2000, which is herein incorporated by reference in its entirety.

Field of the Invention

[0002] The invention relates to a human gamma-3 constant domain containing anti-CD20 antibody and more preferably a human gamma 3 anti-CD20 antibody containing the variable regions of RITUXAN®, which is a chimeric anti-human CD20 antigen binding monoclonal antibody, that is clinically approved for treatment of non-Hodgkin's lymphoma. The invention also relates to therapeutic uses thereof, especially for treating diseases wherein depletion, apoptosis and lysis of CD20 antigen bearing cells is therapeutically beneficial.

Background of the Invention

[0003] The CD20 antigen (also called tumor B-lymphocyte – restricted differentiation antigen, Bp35) is a hydrophobic transmembrane protein having a molecular weight of about 35K located on pre-B and mature B lymphocytes (Valentine et al., *J. Biol. Chem* 264 (14): 11282-11287 (1987); and Einfeldt et al., *EMBOJ.* 7(3): 711-717 (1988)). This antigen is also expressed on greater than 90% of B cell non-Hodgkin's lymphomas (NHL's) (Anderson et al, *Blood* 63 (6): 1424-1433 (1984)), but is not found on stem cells, pro-B cells, normal plasma cells or other normal tissues. Tedder et. al, *J. Immunol* 135(2): 973-977 (1985)). CD20 regulates an early step in the activation process for cell cycle initiation and differentiation (Tedder et al., supra) and possibly function as a calcium ion channel (Tedder et. al, *J. Cell Biochem* 140: 195(1990).

[0004] Given the expression of CD20 by B cell lymphomas, this antigen can serve as a candidate for "targeting" of such lymphomas. In essence, such targeting can be generalized as follows: antibodies specific for CD20 surface

antigen on B cells are administered to a patient. These anti-CD20 antibodies specifically bind to the CD20 antigen of (ostensibly) both normal and malignant B cells, and the antibody bound to the CD20, on the cell surface results in the destruction and depletion of tumorigenic B cells. Additionally, chemical agents, cytotoxins or radioactive agents may be directly or indirectly attached to the anti-CD20 antibody such that the agent is selectively “delivered” to the CD20 antigen expressing B cells. By both of these approaches, the primary goal is to destroy the tumor. The specific approach will depend upon the particular anti-CD20 antibody that is utilized. Thus, it is apparent that the various approaches for targeting the CD20 antigen can vary considerably.

[0005] The rituximab (RITUXAN®) antibody is a genetically engineered chimeric human gamma 1 murine constant domain containing monoclonal antibody directed against the human CD20 antigen. As mentioned, this chimeric antibody contains human gamma 1 constant domains and is identified by the name “C2B8” in US Patent No. 5,736,137 (Andersen et. al.) issued on April 17, 1998, assigned to IDEC Pharmaceuticals Corporation. RITUXAN® is approved for the treatment of patient with relapsed or refracting low-grade or follicular, CD20 positive, B cell non-Hodgkin’s lymphoma. In vitro mechanism of action studies have shown that RITUXAN® exhibits human complement – dependent cytotoxicity (CDC) (Reff et. al, *Blood* 83(2): 435-445 (1994)). Additionally, it exhibits significant activity in assays that measure antibody – dependent cellular cytotoxicity (ADCC). RITUXAN® has been shown to possess anti-proliferative activity in thymidine incorporation assays and to induce apoptosis directly , whereas CD20 antibodies do not [Maloney et. al, *Blood* 88 (10): 637a (1996)].

[0006] Further, it has been shown that synergistic therapeutic benefits are obtained when RITUXAN® is combined with radioimmunotherapy, toxins or chemotherapy. In particular, RITUXAN® sensitizes drug-resistant human B cell lymphoma cell lines to the cytotoxic effects of doxorubicin, CDDP, VP-16, diphtheria toxin and ricin [Demidem et. al, *Cancer Chemotherapy and Radiopharmaceuticals* 12 (3): 177-186 (1997)]. In vitro pre-clinical studies

[0011] More specifically, the invention relates to pharmaceutical compositions containing a chimeric anti-human CD20 antibody comprising the variable regions of RITUXAN® and human gamma 3 constant domains.

[0012] The invention also relates to the use of a human gamma 3 constant domain containing anti-human CD20 antibody as a therapeutic, especially for treatment of diseases wherein deletion, depletion and/or apoptosis of CD20 antigen expressing cells is therapeutically beneficial. Preferably, the antibody will comprise a human gamma 3 constant domain version of RITUXAN®.

[0013] More specifically, the invention relates to the use of a human gamma 3 constant domain containing anti-human CD20 monoclonal antibody for the treatment of a disease selected from the group consisting of B cell lymphomas, leukemias, myelemas, autoimmune disease, transplant, graft-vs-host disease, infectious diseases involving B cells, lymphoproliferation diseases, and treatment of any disease or condition wherein suppression of B cell activity and/or humoral immunity is desirably suppressed.

[0014] More specifically, the invention relates to the use of a human gamma 3 version of RITUXAN® for treatment of a disease selected from the group consisting of B cell lymphomas, leukemia, myeloma, transplant, graft-vs-host disease, autoimmune disease, lymphoproliferation conditions, and other treatment diseases and conditions wherein the inhibition of humoral immunity, B cell function, and/or proliferation, is therapeutically beneficial.

Detailed Description of the Invention

[0015] The present invention in its broadest embodiment relates to chimeric, human or humanized anti-CD20 monoclonal antibodies of the IgG3 isotype, which contain variable regions specific to human CD20 and IgG3 constant domains of human origin having therapeutic activity in naked or unconjugated form. In the preferred embodiment, the anti-CD20 variable regions will be derived from RITUXAN®.

[0016] The "CD20" antigen is a -35 kDa, non-glycosylated phosphoprotein found on the surface of greater than 90% of B cells from peripheral blood or lymphoid organs. CD20 is expressed during early pre-B cell development and

remains until plasma cell differentiation. CD20 is present on both normal B cells as well as malignant B cells. Other names for CD20 in the literature include "B-lymphocyte-restricted antigen" and "Bp35". The CD20 antigen is described in Clark *et al.* *PNAS (USA)* 82:1766 (1985), for example.

[0017] B cell lymphomas in the present invention include any lymphoma involving B cells. Examples thereof include by way of non-Hodgkin's lymphomas of different grades, e.g. low-grade, intermediate-grade, and high-grade, including mixed and large cell lymphoma, Burkitt's lymphoma, diffuse small non-cleaved cell lymphoma, lymphoblastic lymphoma, mantle cell lymphoma, AIDS-related lymphoma, leukemias such as chronic lymphocyte leukemia, and other B cell associated leukemias.

[0018] A "therapeutically effective IgG3 anti-CD20 antibody" according to the invention is a molecule which, upon binding to CD20 antigen destroys or depletes B cells in a mammal and/or interferes with one or more B cell functions, e.g. by reducing or preventing a humoral response elicited by the B cell. The antibody is able to deplete B cells (*i.e.* reduce circulating B cell levels) in a mammal treated therewith. Such depletion may be achieved via various mechanisms such antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC), inhibition of B cell proliferation and/or induction of B cell death (e.g. via apoptosis).

[0019] "Antibody-dependent cell-mediated cytotoxicity" and "ADCC" refer to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) (e.g. Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. The primary cells for mediating ADCC, NK cells, express FcγRI only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or

additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in a animal model such as that disclosed in Clynes *et al. PNAS (USA)* 95:652-656 (1998).

[0020] "Human effector cells" are leukocytes which express one or more FcRs and perform effector functions. Preferably, the cells express at least FcγRIII and carry out ADCC effector function. Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils; with PBMCs and NK cells being preferred.

[0021] The terms "Fc receptor" or "FcR" are used to describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (see Da~ron, *Annu. Rev. Immunol.* 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991); Capel *et al., Immunomethods* 4:25-34 (1994); and de Haas *et al., J. Lab. Clin. Med.* 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer *et al., J. Immunol.* 117:587 (1976) and Kim *et al., J. Immunol.* 24:249 (1994)).

[0022] "Complement dependent cytotoxicity" or "CDC" refer to the ability of a molecule to lyse a target in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (C1q) to a molecule (e.g. an antibody) complexed with a

cognate antigen. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996), may be performed.

[0023] “Growth inhibitory” antibodies are those which prevent or reduce proliferation of a cell expressing an antigen to which the antagonist binds. For example, the antibody may prevent or reduce proliferation of B cells *in vitro* and/or *in vivo*.

[0024] Antibodies or other molecules which “induce apoptosis” are those which induce programmed cell death, e.g. of a B cell, as determined by standard apoptosis assays, such as binding of annexin V, fragmentation of DNA, cell shrinkage, dilation of endoplasmic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies).

[0025] The term “antibody” herein is used in the broadest sense and specifically covers intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity.

[0026] “Antibody fragments” comprise a portion of an intact antibody, preferably comprising the antigen-binding or variable region thereof. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

[0027] “Native antibodies” are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (VH) followed by a number of constant domains. Each light chain has a variable domain at one end (VD) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light-chain

variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

[0028] The term “variable” refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FRs). The variable domains of native heavy and light chains each comprise four ERs, largely adopting a sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the α -sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

[0029] Papain digestion of antibodies produces two identical antigen-binding fragments, called “Fab” fragments, each with a single antigen-binding site, and a residual “Fc” fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-binding sites and is still capable of cross-linking antigen.

[0030] “Fv” is the minimum antibody fragment which contains a complete antigen-recognition and antigen-binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three hypervariable regions of each variable domain interact to define an antigen-binding site on the surface

of the VH-VL dimer. Collectively, the six hypervariable regions confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0031] The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear at least one free thiol group. F(ab')₂ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0032] The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

[0033] Depending on the amino acid sequence of the constant domain of their heavy chains, antibodies can be assigned to different classes. There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy-chain constant domains that correspond to the different classes of antibodies are called α, δ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0034] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody

preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.*, *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (see, *e.g.*, U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, *Nature*, 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991), for example.

[0035] The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in human antibodies derived from a human, particularly those of human IgG3 [U.S. Patent No. 4,816,567; Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)]. Chimeric antibodies of interest herein include "primatized" antibodies comprising variable domain antigen-binding sequences derived from a non-human primate (*e.g.* Old World Monkey, such as baboon, rhesus or cynomolgus monkey) and human constant region sequences (US Pat No. 5,693,780), *i.e.* human IgG3 constant regions.

[0036] "Humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable

[0038] A therapeutically active anti-CD20 antibody, is one capable of binding that antigen with sufficient affinity and/or avidity such that the antibody is useful as a therapeutic agent for targeting a cell expressing the CD20 antigen, e.g. a B cell.

[0039] Examples of known antibodies which bind the CD20 antigen include: “C2B8” which is now called “rituximab” (“RITUXAN®”) (US Patent No. 5,736,137, expressly incorporated herein by reference); the yttrium-[9011-labeled 2B8 murine antibody designated “Y2B8” (US Patent No. 5,736,137, expressly incorporated herein by reference); murine IgG2a “B1” optionally labeled with 1311 to generate the “¹³¹I-B 1” antibody (BEXXARTM) (US Patent No. 5,595,721, expressly incorporated herein by reference); murine monoclonal antibody “1RS” (Press *et al. Blood* 69(2):584-591 (1987)); and “chimeric 2H17” antibody (US Patent No. 5,677,180, expressly incorporated herein by reference).

[0040] The terms “rituximab” or “RITUXAN®” herein refer to the genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen and designated “C2B8” in US Patent No. 5,736,137, expressly incorporated herein by reference. The antibody is an IgG₁ kappa immunoglobulin containing murine light and heavy chain variable region sequences and human constant region sequences. Rituximab has a binding affinity for the CD20 antigen of approximately 8.0nM.

[0041] “Mammal” for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, *etc.* Preferably, the mammal is human.

[0042] “Treatment” refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disease or disorder as well as those in which the disease or disorder is to be prevented. Hence, the mammal may have been diagnosed as having the disease or disorder or may be predisposed or susceptible to the disease.

[illegible]

[0045] The term “cytotoxic agent” as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g. At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³² and radioactive isotopes of Lu), chemotherapeutic agents, and toxins such as small molecule toxins or

enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

[0046] A “chemotherapeutic agent” is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide (CYTOXANTM); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziidines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelarnine, trietylenephosphoramidate, triethylenethiophosphoramidate and trimethylolomelamine; nitrogen mustards such as chiorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfarnide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabycin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-EU; androgens such as calusterone, dromostanolone propionate, epitostanol, mepitostane, testolactone; anti-adrenal such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan;

[illegible]

[0048] The term “prodrug” as used in this application refers to a precursor or derivative form of a pharmaceutically active substance that is less cytotoxic to tumor cells compared to the parent drug and is capable of being enzymatically activated or converted into the more active parent form. See, e.g., Wilman, “Prodrugs in Cancer Chemotherapy” *Biochemical Society Transactions*, 14, pp. 375-382, 6 15th Meeting Belfast (1986) and Stella *et al.*, “Prodrugs: A Chemical Approach to Targeted Drug Delivery,” *Directed Drug Delivery*, Borchardt *et al.*, (ed.), pp. 247-267, Humana Press (1985). The prodrugs of this invention include, but are not limited to, phosphate-containing prodrugs, thiophosphate-containing prodrugs, sulfate-containing prodrugs, peptide-containing prodrugs, D-amino acid-modified prodrugs, glycosylated prodrugs, β -lactam-containing prodrugs, optionally substituted phenoxyacetamide-containing prodrugs or optionally substituted phenylacetamide-containing prodrugs, 5-fluorocytosine and other 5-fluorouridine prodrugs which can be converted into the more active cytotoxic free drug. Examples of cytotoxic drugs that can be derivatized into a prodrug form for use in this invention include, but are not limited to, those chemotherapeutic agents described above.

[0049] A “liposome” is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as the antagonists disclosed herein and, optionally, a chemotherapeutic agent) to

a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

[0050] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products.

1. Production of Anti-DC20 Antibodies to the Invention.

[0051] The methods and articles of manufacture of the present invention use, or incorporate, a IgG3 anti monoclonal antibody which binds human CD20 antigen containing human IgG3 constant domains. Accordingly, methods for generating such antibodies will be described here.

[0052] A description follows as to exemplary techniques for the production of antibodies produced in accordance with the present invention.

A. Polyclonal antibodies

[0053] Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, *e.g.*, keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosucciniride ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl_2 , or $\text{R}^1\text{N}=\text{C}=\text{NR}$, where R and R^1 are different alkyl groups.

[0054] Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, *e.g.*, 100 μg or 5 μg of the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the

serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same-antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

B. Monoclonal antibodies

[0055] Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies.

[0056] For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohier *et al.*, *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

[0057] In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)).

[0058] The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

[0063] DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra-*et al.*, *Curr. Opinion in Immunol.*, 5:256-262 (1993) and Plückthun, *Immunol. Revs.*, 130:151-188 (1992).

[0064] In a further embodiment, antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty *et al.*, *Nature*, 348:552-554 (1990). Clackson *et al.*, *Nature*, 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks *et al.*, *Bio/Technology*, 10:779-783 (1992)), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries (Waterhouse *et al.*, *Nuc. Acids. Res.*, 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

[0065] The DNA is modified by substituting the coding sequence for human 1gG3 heavy-and light-chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison, *et al.*, *Proc. Natl Acad. Sci. USA*, 81:6851 (1984)).

C. Humanized Anti-CD20 antibodies

[0066] Methods for humanizing non-human antibodies are known in the art. Preferably, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human

amino acid residues are often referred to as “import” residues, which are typically taken from an “import” variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.*, *Nature*, 321:522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-327 (1988); Verhoeven *et al.*, *Science*, 239:1534-1536 (1988)), by substituting hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such “humanized” antibodies are chimeric antibodies (U.S. Patent No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies. The subject humanized anti-CD20 antibodies will comprise constant regions of human IgG3.

[0067] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called “best-fit” method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework region (FR) for the humanized antibody (Sims *et al.*, *J. Immunol.*, 151:2296 (1993); Chothia *et al.*, *J. Mol. Biol.*, 196:901 (1987)). Another method uses a particular framework region derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); Presta *et al.*, *J. Immunol.*, 151:2623 (1993)).

[0068] It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the

parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

D. Human antibodies

[0069] As an alternative to humanization, human anti-CD20, antibodies can be generated. For example, it is now possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, *e.g.*, Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:2551 (1993); Jakobovits *et al.*, *Nature*, 362:255-258 (1993); Bruggermann *et al.*, *Year in Immuno.*, 7:33 (1993); and US Patent Nos. 5,591,669, 5,589,369 and 5,545,807.

[0070] Alternatively, phage display technology (McCafferty *et al.*, *Nature* 348:552-553 (1990)) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional

antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B cell. Phage display can be performed in a variety of formats; for their review see, e.g., Johnson, Kevin S. and Chiswell, David J., *Current Opinion in Structural Biology* 3:564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson *et al.*, *Nature*, 352:624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991), or Griffith *et al.*, *EMBO J.* 12:725-734 (1993). See, also, US Patent Nos. 5,565,332 and 5,573,905.

[0071] Human antibodies may also be generated by *in vitro* activated B cells (see US Patents 5,567,610 and 5,229,275). Human antibodies according to the invention will be selected that contain 1gG3 human constant domains.

[0072] The therapeutic use of IgG1 monoclonal anti-human CD20 antibodies, especially for killing or modulating cells bearing the CD20 antigen is disclosed in US Patent 5,721,108 and 5,500,362, issued to Robinson, et al, and assigned to XOMA Corporation. However, the disclosed antibodies possess IgG1 rather than IgG3 effector functions. In fact, the therapeutic activity of these antibodies is disclosed to reside in their ability to induce antibody – dependent cellular cytotoxicity (ADCC) or complement dependent (CDC), based on the effector function of the IgG1 human constant domains.

[0073] The fact that the therapeutic activity is reportedly attributable to the selection of human IgG1 or constant domain is evidenced, e.g. based on various assays disclosed in US Patent 5,721,108 which compares a chimeric IgG1 anti-CD30 antibody 2H7 (containing human IgG1 constant domains) with respect to its ability to elicit ADCC and CDC activity relative to a parent

murine anti-CD20 antibody 2H7 antibody (containing mouse IgG3 constant regions). The patentees teach, based on their results, that chimeric antibodies will be suitable for treating B cell disorders, especially leukemias and lymphomas.

[0074] A preferred and well known example of a chimeric IgG1 anti-human CD20 antibody that possesses both ADCC and CDC activity is RITUXAN®, which is the first antibody approved for treatment of monoclonal cancer (non-Hodgkin's lymphoma).

[0075] The synthesis of this antibody and description of this antibody including the complete amino acid and DNA sequence of the variable heavy and light chains of RITUXAN® is contained in US Patents 5,736,137; 5,776,456; and 5,843,343, issued to Anderson et al and assigned to IDEC Pharmaceutical Corporation. These patents are incorporated by reference in their entirety herein.

[0076] As noted previously, RITUXAN® has been approved for treatment of non-Hodgkin's lymphomas. In fact, RITUXAN® is the first antibody approved for use in the treatment of human cancer.

[0077] RITUXAN®, which has been demonstrated to possess substantial B cell depleting activity, complement dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), and CD20 expressing cells. However, it has not been reported that anti-human CD20 antibodies containing human IgG3 constant domains possess similar activity.

[0078] Also, it has been demonstrated that RITUXAN® possesses anti-proliferation activity in thymidine incorporation assays and induces apoptosis directly, whereas other anti-CD20 antibodies do not (Maloney et al, *Id.*).

[0079] According to the present invention, human, chimeric or humanized IgG3 anti-human CD20 antibodies containing human IgG3 constant domains will be selected that exhibit at least one of ADCC activity, CDC activity, ability to deplete CD20 antigen expressing cells, or to induce the apoptosis of CD20 antigen expressing cells. Preferably, such chimeric, humanized or human IgG3 antibodies will comprise at least 25%, more preferably at least 50%, and even more preferably at least 90% of the ADCC, CDC, apoptosis activity of a

comparable anti-human CD20 antibody differing only in the fact that it contains human IgG1 rather than IgG3 constant domains.

2. Conjugates and Other Modifications of the Subject IgG3 Anti-CD20 Antibodies

[0080] The antibodies used in the methods or included in the articles of manufacture herein are optionally conjugated to a toxin, drug or chemotherapeutic.

[0081] Chemotherapeutic agents useful in the generation of such antagonist-cytotoxic agent conjugates have been described above.

[0082] Conjugates of the subject antibody and one or more small molecule toxins, such as a calicheamicin, a maytansine (US Patent No. 5,208,020), a trichothene, and CC1065 are also contemplated herein. In one embodiment of the invention, the antibody is conjugated to one or more maytansine molecules (e.g. about 1 to about 10 maytansine molecules per antagonist molecule). Maytansine may, for example, be converted to May-SS-Me which may be reduced to May-SH3 and reacted with modified antibody [Chari *et al.*, *Cancer Research* 52:127-131 (1992)] to generate a maytansinoid-antagonist conjugate.

[0083] Alternatively, the antibody is conjugated to one or more calicheamicin molecules. The calicheamicin family of antibiotics are capable of producing double-stranded DNA breaks at sub-picomolar concentrations. Structural analogues of calicheamicin which may be used include, but are not limited to, γ_1^I , α_2^I , α_3^I , N-acetyl- γ_1^I , PSAG and θ^I_1 (Hinman *et al.* *Cancer Research* 53: 3336-3342 (1993) and Lode *et al.* *Cancer Research* 58: 2925-2928 (1998)).

[0084] Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleuritesfordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPH, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor,

gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, for example, WO 93/21232 published October 28, 1993.

[0085] The present invention further contemplates the antibody conjugated with a compound with nucleolytic activity (e.g. a ribonuclease or a DNA endonuclease such as a deoxyribonuclease; DNase).

[0086] A variety of radioactive isotopes are available for the production of radioconjugated antagonists. Examples include At^{211} , I^{131} , I^{125} , Y^{90} , Re^{186} , Re^{188} , Sm^{153} , Bi^{212} , P^{32} and radioactive isotopes of Lu.

[0087] Conjugates of the antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate, iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al. Science* 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antagonist. See WO94/11026. The linker may be a "cleavable linker" facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, dimethyl linker or disulfide-containing linker (Charm *et al. Cancer Research* 52: 127-131 (1992)) may be used.

[0088] Alternatively, a fusion protein comprising the antibody and cytotoxic agent may be made, e.g. by recombinant techniques or peptide synthesis. In yet another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antagonist-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then

administration of a "ligand" (e.g. avidin) which is conjugated to a cytotoxic agent (e.g. a radionucleotide).

[0089] The IgG3 antibodies of the present invention may also be conjugated with a prodrug-activating enzyme which converts a prodrug (e.g. a peptidyl chemotherapeutic agent, see WO81/01145) to an active anti-cancer drug. See, for example, WO 88/07378 and U.S. Patent No. 4,975,278.

[0090] The enzyme component of such conjugates includes any enzyme capable of acting on a prodrug in such a way so as to convert it into its more active, cytotoxic form.

[0091] Enzymes that are useful in the method of this invention include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β -galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs; β -lactamase useful for converting drugs derivatized with β -lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (see, e.g., Massey, *Nature* 328: 457-458 (1987)). Antagonist-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.

[0092] The enzymes of this invention can be covalently bound to the subject IgG3 antibody by techniques well known in the art such as the use of the heterobifunctional crosslinking reagents discussed above. Alternatively,

fusion proteins comprising at least the antigen binding region of an antagonist of the invention linked to at least a functionally active portion of an enzyme of the invention can be constructed using recombinant DNA techniques well known in the art (see, e.g., Neuberger *et al.*, *Nature*, 312: 604-608 (1984)).

[0093] Other modifications of the antibody are contemplated herein. For example, the antibody may be linked to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol.

[0094] The antibodies disclosed herein may also be formulated as liposomes. Liposomes containing the antagonist are prepared by methods known in the art, such as described in Epstein *et al.*, *Proc. Natl. Acad. Sci. USA*, 82:3688 (1985); Hwang *et al.*, *Proc. Natl. Acad. Sci. USA*, 77:4030 (1980); U.S. Pat. Nos. 4,485,045 and 4,544,545; and WO97/38731 published October 23, 1997. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556. Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of an antibody of the present invention can be conjugated to the liposomes as described in Martin *et al.* *J. Biol. Chem.* 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent is optionally contained within the liposome. See Gabizon *et al.* *J. National Cancer Inst.* 81(19) 1484 (1989).

[0095] Amino acid sequence modification(s) of the IgG3 anti-CD20 antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antagonist. Amino acid sequence variants of the antagonist are prepared by introducing appropriate nucleotide changes into the antagonist nucleic acid, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antagonist. Any combination of deletion,

insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the antagonist, such as changing the number or position of glycosylation sites.

[0096] A useful method for identification of certain residues or regions of the antagonist that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells *Science*, 244:1081-1085 (1989). Here, a residue or group of target residues are identified (e.g., charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed antagonist variants are screened for the desired activity.

[0097] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antagonist with an N-terminal methionyl residue or the antagonist fused to a cytotoxic polypeptide. Other insertional variants of the antagonist molecule include the fusion to the N- or C-terminus of the antagonist of an enzyme, or a polypeptide which increases the serum half-life of the antagonist.

[0098] Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the antagonist molecule replaced by different residue. The sites of greatest interest for substitutional mutagenesis of antibody antagonists include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown

in Table 1 under the heading of "preferred substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in Table 1, or as further described below in reference to amino acid classes, may be introduced and the products screened.

Table 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	val; leu; ile	Val
Arg (R)	lys; gln; asn	Lys
Asn (N)	gln; his; asp, lys; arg	Gln
Asp (D)	glu; asn	Glu
Cys (C)	ser; ala	Ser
Gln (Q)	asn; glu	Asn
Glu (E)	asp; gln	Asp
Gly (G)	ala	Ala
His (H)	asn; gln; lys; arg	Arg
Ile (I)	leu; val; met; ala; phe; norleucine	Leu
Leu (L)	norleucine; ile; val; met; ala; phe	Ile
Lys (K)	arg; gln; asn	Arg
Met (M)	meu; phe; ile	Leu
Phe (F)	leu; val; ile; ala; tyr	Tyr
Pro (P)	ala	Ala
Ser (S)	thr	Thr
Thr (T)	ser	Ser
Trp (W)	try; phe	Tyr
Tyr (Y)	trp; phe; thr; ser	Phe
Val (V)	ile; leu; met; phe; ala; norleucine	Leu

[0099] Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

hydrophobic: norleucine, met, ala, val, leu, ile;

neutral hydrophilic: cys, ser, thr;

acidic: asp, glu;

basic: asn, gln, his, lys, arg;

residues that influence chain orientation: gly, pro; and

aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0100] Any cysteine residue not involved in maintaining the proper conformation of the antagonist also may be substituted, generally with seine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antagonist to improve its stability (particularly where the antagonist is an antibody fragment such as an Fv fragment).

[0101] A particularly preferred type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody. Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants is affinity maturation using phage display. Briefly, several hypervariable region sites (e.g. 6-7 sites) are mutated to generate all possible amino substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed

variants are then screened for their biological activity (e.g. binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or in additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

[0102] Another type of amino acid variant of the antibody alters the original glycosylation pattern of the antibody. By altering is meant deleting one or more carbohydrate moieties found in the antagonist, and/or adding one or more glycosylation sites that are not present in the antibody.

[0103] Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[0104] Addition of glycosylation sites to the antagonist is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antagonist (for O-linked glycosylation sites).

[0105] Nucleic acid molecules encoding amino acid sequence variants of the antibody are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antagonist.

[0106] It may be desirable to modify the subject IgG3 antibody to further enhance effector function, e.g. so as to enhance antigen-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) of the antagonist. This may be achieved by introducing one or more amino acid substitutions in the Fc region of an antibody antagonist. Alternatively or additionally, cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, *J. Exp Med.* 176:1191-1195 (1992) and Shopes, B. *J. Immunol.* 148:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al.* *Cancer Research* 53:2560-2565 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al.* *Anti-Cancer Drug Design* 3:219-230 (1989).

[0107] To increase the serum half life of the antibody, one may incorporate a salvage receptor binding epitope into the antagonist (especially an antibody fragment) as described in US Patent 5,739,277, for example. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (e.g., IgG₁, IgG₂, IgG₃, or IgG₄) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule.

3. Pharmaceutical Formulations

[0108] Therapeutic formulations containing the subject IgG3 anti-human CD20 monoclonal antibodies are prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[0109] Exemplary anti-CD20 antibody formulations are described in WO98/56418, expressly incorporated herein by reference. This publication describes a liquid multidose formulation comprising 40 mg/mL rituximab, 25 mM acetate, 150 mM trehalose, 0.9% benzyl alcohol, 0.02% polysorbate 20 at pH 5.0 that has a minimum shelf life of two years storage at 2-8 °C. Another anti-CD20 formulation of interest comprises 10mg/mL rituximab in 9.0 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7mg/mL polysorbate 80, and Sterile Water for Injection, pH6.5. In the present invention,

RITUXAN® will be substituted by an IgG3 anti-human CD20 monoclonal antibody.

[0110] Lyophilized formulations adapted for subcutaneous administration are described in WO97/04801. Such lyophilized formulations may be reconstituted with a suitable diluent to a high protein concentration and the reconstituted formulation may be administered subcutaneously to the mammal to be treated herein.

[0111] The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide a cytotoxic agent, chemotherapeutic agent, cytokine or immunosuppressive agent (e.g. one which acts on T cells, such as cyclosporin or an antibody that binds T cells, e.g. one which binds LFA-1). The effective amount of such other agents depends on the amount of antagonist present in the formulation, the type of disease or disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as used hereinbefore or about from 1 to 99% of the heretofore employed dosages.

[0112] The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

[0113] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antagonist, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-

hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

[0114] The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

4. Treatment with the Subject IgG3 Anti-CD20 Monoclonal Antibodies

[0115] The composition comprising an anti-human CD20 monoclonal antibody containing human IgG3 constant domains will be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disease or disorder being treated, the particular mammal being treated, the clinic condition of the individual patient, the cause of the disease or disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The therapeutically effective amount of the antagonist to be administered will be governed by such considerations.

[0116] As a general proposition, the therapeutically effective amount of the antibody administered parenterally per dose will be in the range of about 0.1 to 20 mg/kg of patient body weight per day, with the typical initial range of antagonist used being in the range of about 2 to 10 mg/kg.

[0117] The preferred antibody, will contain the variable regions of RITUXAN® and human IgG3 constant regions and will not be conjugated to a cytotoxic agent. Suitable dosages for an unconjugated antibody are, for example, in the range from about 20mg/m² to about 1000mg/m². In one embodiment, the dosage of the antibody differs from that presently recommended for RITUXAN®. For example, one may administer to the patient one or more doses of substantially less than 375mg/m² of the

antibody, e.g. where the dose is in the range from about 20mg/m² to about 250mg/m², for example from about 50mg/m² to about 200mg/m².

[0118] Moreover, one may administer one or more initial dose(s) of the antibody followed by one or more subsequent dose(s), wherein the mg/m² dose of the antibody in the subsequent dose(s) exceeds the mg/m² dose of the antibody in the initial dose(s). For example, the initial dose may be in the range from about 20mg/m² to about 250mg/m² (e.g. from about 50mg/m² to about 200mg/m²) and the subsequent dose may be in the range from about 250mg/m² to about 1000mg/m².

[0119] As noted above, however, these suggested amounts of antibody are subject to a great deal of therapeutic discretion. The key factor in selecting an appropriate dose and scheduling is the result obtained, as indicated above. For example, relatively higher doses may be needed initially for the treatment of ongoing and acute diseases. To obtain the most efficacious results, depending on the disease or disorder, the antagonist is administered as close to the first sign, diagnosis, appearance, or occurrence of the disease or disorder as possible or during remissions of the disease or disorder.

[0120] The anti-CD20 monoclonal antibody is administered by any suitable means, including parenteral, subcutaneous, intraperitoneal, intrapulmonary, and intranasal, and, if desired for local immunosuppressive treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. In addition, the antagonist may suitably be administered by pulse infusion, e.g., with declining doses of the antagonist. Preferably the dosing is given by injections, most preferably intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic.

[0121] One may administer other compounds, such as cytotoxic agents, chemotherapeutic agents, immunosuppressive agents and/or cytokines with the antagonists herein. The combined administration includes coadministration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably

there is a time period while both (or all) active agents simultaneously exert their biological activities.

5. Articles of Manufacture

[0122] In another embodiment of the invention, an article of manufacture containing materials useful for the treatment of the diseases or disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds or contains a composition which is effective for treating the disease or disorder of choice and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one antibody according to the invention is contained in the composition. The label or package insert indicates that the composition is used for treating a patient having or predisposed to a disease that is treatable with an anti-CD20 antibody according to the invention. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable diluent buffer, such as bacteriostatic wat for injection (BWFI), phospho - buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0123] Further details of the invention are illustrated by the following non-limiting Examples. The disclosures of all citations in the specification are expressly incorporated herein by reference.

6. Therapeutic Utility

[0124] The subject IgG3 anti-human CD20 monoclonal antibodies are useful as therapeutics or prophylactives for the treatment/prophylaxis of any disease or condition wherein the modulation, deletion, depletion, and/or

apoptosis of CD20 antigen expressing cells are therapeutically or prophylactically beneficial.

[0125] Such conditions include in particular B cell lymphomas and leukemias including by way of example non-Hodgkins lymphomas, chronic lymphocytic leukemias and other lymphomas previously identified.

[0126] Also such conditions include diseases and treatments wherein inhibition of humoral immunity is therapeutically beneficial. Examples of such diseases include autoimmune diseases, transplant, graft-vs-host diseases, host-vs-graft diseases, cell therapy, gene therapy, therapy involving the administration of potentially antigenic drugs, e.g. antigenic proteins such as therapeutic antibodies.

[0127] Also, the subject IgG3 anti-human CD20 antibodies may be combined with other therapies that induce apoptosis or the killing of B cells, e.g. external beam radiation, and other antibodies, preferably others that specifically bind antigens expressed on B cells, such as B7.1 (CD80), B7.2 (CD86), CD19, CD21, CD22, CD23, CD37, CD40, etc., which may be radiolabeled or attached to chemotherapeutics.

[0128] In order to more clearly describe the invention, the following examples are provided.

EXAMPLES

Example 1

[0129] A chimeric IgG3 anti-human CD20 antibody containing the variable heavy and variable light regions of RITUXAN® (disclosed in US Patents 5736137; 5,776,456 and 5,843,437, assigned to IDEC Pharmaceuticals Corporation) is produced (which is identical to RITUXAN®, except that the IgG1 human constant domains are substituted with human IgG3 constant domains).

[0130] Nucleic acid sequences encoding human IgG3 constant domains can be obtained from human IgG3 producing cells by standard cloning techniques. The subject chimeric IgG3 anti-CD20 antibody is preferably expressed using IDEC's proprietary expression vector system known as

TCAE which provides for co-expression of variable light and variable heavy regions fused to IgG3 human heavy and light constant domains. This vector system contains a translationally impaired neo-gene that provides for enhanced high antibody expression, and is disclosed in U.S. Patent 5,648,267 incorporated by reference in its entirety herein.

Example 2

[0131] The chimeric IgG3 anti-human CD20 antibody produced according to example 1, which contains the variable heavy and light regions of RITUXAN® and human IgG3 constant regions is tested *in vitro* for its ability to induce ADDC and CDC activity. Further, this monoclonal antibody is tested for its ability to inhibit the proliferation of human B cell lymphoma cells in vitro by inducing apoptosis.

[0132] An assay measures the ability of this antibody to inhibit thymidine incorporation and to induce apoptosis directly and is as disclosed in Reff et al., *Blood* 88(10): 637a (1996).

Example 3

[0133] Patients with clinical diagnosis of rheumatoid arthritis (RA) are treated with a chimeric IgG3 monoclonal anti-CD20 antibody containing the variable regions of RITUXAN®) antibody. Moreover, the patient is optionally further treated with any one or more agents employed for treating RA such as salicylate; nonsteroidal anti-inflammatory drugs such as indomethacin, phenylbutazone, phenylacetic acid derivatives (e.g. ibuprofen and fenoprofen), naphthalene acetic acids (naproxen), pyrrolealkanoic acid (tometin), indoleacetic acids (sulindac), halogenated anthranilic acid (meclofenamate sodium), piroxicam, zomepirac and diflunisal; antimalarials such as chloroquine; gold salts; penicillamine; or immunosuppressive agents such as methotrexate or corticosteroids in dosages known for such drugs or reduced dosages. Preferably however, the patient is only treated with RITUXAN®.

[0134] Chimeric IgG3 anti-human CD20 monoclonal antibody is administered intravenously (IV) to the patient according to any of the following dosing schedules:

50mg/m² IV day 1
150 mg/m² IV on days 8, 15 & 22
150mg/m² IV day 1
375 mg/m² IV on days 8, 15 & 22
375 mg/m² IV days 1, 8, 15 & 22

The primary response is determined by the Paulus index (Paulus *et al. Arthritis Rheum.* 33:477-484 (1990)), i.e. improvement in morning stiffness, number of painful and inflamed joints, erythrocyte sedimentation (ESR), and at least a 2-point improvement on a 5-point scale of disease severity assessed by patient and by physician. Administration of the subject IgG3 anti-human CD20 antibody will alleviate one or more of the symptoms of RA in the patient treated as described above.

Example 4

[0135] Patients diagnosed with autoimmune hemolytic anemia (AIHA), e.g., cryoglobulinemia or Coombs positive anemia, are treated with chimeric IgG3 anti-CD20 antibody. AIHA is an acquired hemolytic anemia due to auto-antibodies that react with the patient's red blood cells.

[0136] Chimeric IgG3 anti-human CD20 antibody is administered intravenously (IV) to the patient according to any of the following dosing schedules:

50mg/m² IV day I
150mg/m² IV on days 8, 15 & 22
150mg/m² IV day I
375 mg/m² IV on days 8, 15 & 22
375 mg/m² IV days 1, 8, 15 & 22

Further adjunct therapies (such as glucocorticoids, prednisone, azathioprine, cyclophosphamide, vinca-laden platelets or Danazol) may be combined with

the CD20 antibody, but preferably the patient is treated with the subject IgG3 anti-CD20 antibody as a single-agent throughout the course of therapy.

[0137] Overall response rate is determined based upon an improvement in blood counts, decreased requirement for transfusions, improved hemoglobin levels and/or a decrease in the evidence of hemolysis as determined by standard chemical parameters. Administration of the IgG3 anti-CD20 antibody will improve any one or more of the symptoms of hemolytic anemia in the patient treated as described above.

Example 5

[0138] Adult immune thrombocytopenic purpura (ITP) is a relatively rare hematologic disorder that constitutes the most common of the immune-mediated cytopenias. The disease typically presents with severe thrombocytopenia that may be associated with acute hemorrhage in the presence of normal to increased megakaryocytes in the bone marrow. Most patients with ITP have an IgG antibody directed against target antigens on the outer surface of the platelet membrane, resulting in platelet sequestration in the spleen and accelerated reticuloendothelial destruction of platelets (Bussell, J.B. *Hematol. Oncol. Clin. North Am.* (4):179 (1990)). A number of therapeutic interventions have been shown to be effective in the treatment of ITP. Steroids are generally considered first-line therapy, after which most patients are candidates for intravenous immunoglobulin (IVIG), splenectomy, or other medical therapies including vincristine or immunosuppressive/cytotoxic agents. Up to 80% of patients with ITP initially respond to a course of steroids, but far fewer have complete and lasting remissions. Splenectomy has been recommended as standard second-line therapy for steroid failures, and leads to prolonged remission in nearly 60% of cases yet may result in reduced immunity to infection. Splenectomy is a major surgical procedure that may be associated with substantial morbidity (15%) and mortality (2%). IVIG has also been used as second line medical therapy, although only a small proportion of adult patients with ITP achieve remission.

[0139] Therapeutic options that would interfere with the production of autoantibodies by activated B cells without the associated morbidities that occur with corticosteroids and/or splenectomy would provide an important treatment approach for a proportion of patients with ITP.

[0140] Patients with clinical diagnosis of ITP (e.g. with a platelet count <75,000/ μ L) are treated with an IgG3 antibody containing the variable regions of rituximab (RITUXAN®) antibody, optionally in combination with steroid therapy. The patient treated will not have a B cell malignancy.

[0141] A chimeric IgG3 anti-human CD20 monoclonal antibody according to the invention is administered intravenously (IV) to the ITP patient according to any of the following dosing schedules:

50mg/m² IV day 1

150 mg/m² IV on days 8, 15 & 22

150mg/m² IV day 1

375 mg/m² IV on days 8, 15 & 22

375 mg/m² IV days 1, 8, 15 & 22

Patients are premedicated with one dose each of diphenhydramine 25-50 mg intravenously and acetaminophen 650 mg orally prior to the infusion of the antibody. Using a sterile syringe and a 21 gauge or larger needle, the necessary amount of antibody is transferred from the vial into an IV bag containing sterile, pyrogen-free 0.9% Sodium Chloride, USP (saline solution). The final concentration of the subject chimeric antibody is approximately 1 mg/mL. The initial dose infusion rate is initiated at 25 mg/hour for the first half hour then increased at 30 minute intervals by 50 mg/hr increments to a maximum rate of 200 mg/hours. If the first course of antibody is well tolerated, the infusion rates of subsequent courses start at 50 mg/hour and escalate at 30 minute intervals by 100 mg/hour increments to a maximum rate not to exceed 300 mg/hr. Vital signs (blood pressure, pulse, respiration, temperature) are monitored every 15 minutes x 4 or until stable, and then hourly until the infusion is completed.

[0142] Overall response rate is determined based upon a platelet count determined on two consecutive occasions two weeks apart following the four

Country	Year	Value	Unit
Algeria	1980	1.00	1000
Algeria	1981	1.00	1000
Algeria	1982	1.00	1000
Algeria	1983	1.00	1000
Algeria	1984	1.00	1000
Algeria	1985	1.00	1000
Algeria	1986	1.00	1000
Algeria	1987	1.00	1000
Algeria	1988	1.00	1000
Algeria	1989	1.00	1000
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Algeria	2018	1.00	1000
Algeria	2019	1.00	1000
Algeria	2020	1.00	1000
Algeria	2021	1.00	1000
Algeria	2022	1.00	1000
Algeria	2023	1.00	1000
Algeria	2024	1.00	1000
Algeria	2025	1.00	1000
Algeria	2026	1.00	1000
Algeria	2027	1.00	1000
Algeria	2028	1.00	1000
Algeria	2029	1.00	1000
Algeria	2030	1.00	1000
Algeria	2031	1.00	1000
Algeria	2032	1.00	1000
Algeria	2033	1.00	1000
Algeria	2034	1.00	1000
Algeria	2035	1.00	1000
Algeria	2036	1.00	1000
Algeria	2037	1.00	1000
Algeria	2038	1.00	1000
Algeria	2039	1.00	1000
Algeria	2040	1.00	1000
Algeria	2041	1.00	1000
Algeria	2042	1.00	1000
Algeria	2043	1.00	1000
Algeria	2044	1.00	1000
Algeria	2045	1.00	1000
Algeria	2046	1.00	1000
Algeria	2047	1.00	1000
Algeria	2048	1.00	1000
Algeria	2049	1.00	1000
Algeria	2050	1.00	1000
Algeria	2051	1.00	1000
Algeria	2052	1.00	1000
Algeria	2053	1.00	1000
Algeria	2054	1.00	1000
Algeria	2055	1.00	1000
Algeria	2056	1.00	1000
Algeria	2057	1.00	1000
Algeria	2058	1.00	1000
Algeria	2059	1.00	1000
Algeria	2060	1.00	1000
Algeria	2061	1.00	1000
Algeria	2062	1.00	1000
Algeria	2063	1.00	1000
Algeria	2064	1.00	1000
Algeria	2065	1.00	1000
Algeria	2066	1.00	1000
Algeria	2067	1.00	1000

[0143] A patient with non-Hodgkin's lymphoma is treated with a chimeric IgG3 anti-human CD20 antibody according to the invention by the same intravenous protocol described in example 3.

Example 7

[0145] A patient with CLL is treated with a chimeric IgG3 anti-human CD20 antibody according to the invention by the same intravenous protocol described in example 3.

[0147] Although the invention herein has been described in detail with respect to preferred embodiments, other embodiments within the teachings of the present invention are possible. Accordingly, the disclosure should not be limited by the description of the preferred embodiments.